ness landscape of promoter sequences, our result suggested a following image of the landscape: a high sharp peak (corresponding to the final survivor) and a not-so-high but very broad hill, although we need further study to confirm this image. We will also report about the competition experiment between the optimal promoter obtained and the natural $\varphi 2.5$ promoter.

1P284	複数時間スケールを持つ環境適応ダイナミクス：エタノールストレス環境下での大腸菌人工進化実験における網羅的表現型/遺伝子型解析
1H1255	Environmental adaptation over multiple time scales: comprehensive expression/mutation analysis of evolved E. coli under ethanol stress

Takaaki Horinouchi¹, Kuniyasu Tamaoka¹, **Chikara Furusawa¹**, Naoaki Ono¹, Shingo Suzuki¹, Takashi Hirasawa¹, Tetsuya Yomo^{1,2}, Hiroshi Shimizu¹, (¹*Graduate School of Information Science and Technology, Osaka University*; ²*Graduate School of Frontier Biosciences, Osaka University*)

Advances of technologies for comprehensive analysis now make it possible to reveal the phenotypic and genetic changes responsible for the adaptive evolution. Such detailed information of evolutionary traits provides a basis for understanding intra-cellular dynamics with multiple time-scales, including environmental response, adaptation, and evolution. In this study, we performed parallel evolution experiments of *Escherichia coli* under 5% ethanol stress condition. After cultivation of more than 1000 generations (2500 hours), we obtained 6 ethanol-tolerant strains independently, which exhibited about 2 folds increase in specific growth rates compared to the parent strain. We performed comprehensive gene expression analysis of these tolerant strains to analyze phenotypic changes occurred during the adaptive evolution, and found that several gene functions were significantly up- or down-regulated. Furthermore, single-nucleotide substitutions in the tolerant strain's genome fixed during the adaptive evolution were analyzed by microarray-based resequencing experiments, and we found that the number of fixed mutations were less than 10 in some tolerant strains, suggesting that the phenotype of ethanol tolerance was not caused by genomic mutations, instead it was due to environmental adaptation having long time-cale occurred without genomic mutations. Based on these comprehensive phenotypic/genetic data, the relationship between adaptive dynamics over different time scales will be discussed.

1P285	遺伝子型-表現型対応付け戦略から見た初期翻訳系の起源へのアプローチ
	An approach towards the origin of primitive translation system from the viewpoint of genotype-phenotype linking strategy

Naoto Nemoto¹, Yuzuru Husimi², (¹*Department of Functional Materials Science, Saitama University*; ²*Innovative Research Organization, Saitama University*)

As one of the post-genome era problems, an artificial minimum cell has been investigated and recently, the first trial was reported. The minimization problem has had an ambiguity about the setup of the "ecological" environment, especially when one treated a heterotrophic cell. And this ambiguity will become inaccessible in the study of the origins of life. For example, how the translation system was emerged in a cell on the Earth? Eigen's hypercycle theory was a plausible answer to this question [1]. We found that there is an alternative way to introduce the Darwinian evolution into the hypercycle theory from a point of view of genotype-phenotype linking strategy [2]. In order to link DNA or RNA (genotype) to protein (phenotype), there is the virus-type strategy in addition to the cell-type one. In evolutionary molecular engineering, the virus-type strategy (e.g. phage display) is more powerful than the cell-type in the selection of single function of a protein. Thus, we tried to examine which strategy is more efficient in the evolution of a primitive translational activity, using a stochastic mathematical model for a simple virus-like molecule like "*in vitro* virus [3]" and a proto-cell model. We found that the virus-like molecule of which protein binds to its genomic RNA can accelerate the translational activity smoothly in comparison with the proto-cell of which division occurs randomly.

[1] Eigen & Schuster, *The Hypercycle* (1979) [2] Nemoto & Husimi,*J. theor. Biol.*, **176**, 67 (1995) [3] Nemoto,*et al.*, *FEBS lett.* **414**, 405 (1997)

1P286	進化する遺伝情報の自己複製システムの構築
1H1310	Construction of an evolvable self-replication system of genetic information

Norikazu Ichihashi¹, Tomoaki Matsuura¹, Tetsuya Yomo^{1,2,3}, (¹*ERATO JST*; ²*Graduate School of Information Science and Technology, Osaka University*; ³*Graduate School of Frontier Bioscience, Osaka University*)

Evolvability is one of the central characteristics of life. This ability allows a living system to diverse into a vast number of species. Non-living entity

does not have evolvability. How can such ability emerge from non-living molecules? If a system obtains evolvability, does it evolve as an extant life? These questions have not been experimentally investigated. We addressed these questions by constructing an evolvable system. We used a self-replication system of genetic information that was previously constructed *in vitro*. This system is composed of a template RNA (the genetic information) encoding RNA replicase (*Q β* replicase) and a cell-free translation system. We encapsulated the system into micro-sized compartments to link the genotype with phenotype. In addition, we amplified the self-replicated RNA through reverse-transcription, PCR, and *in vitro* transcription. Cycles of these successive reactions enabled the continuous self-replication process. As model experiment, mixture of two template RNAs that have slightly different self-replication activities were subjected into the self-replication-amplification cycles. The result shows that one of the RNA with 1.3 fold higher self-replication activity dominates the population after four rounds of the cycle. This result implies that a mutant RNA with slightly higher self-replication activity can dominate the population after several round of the cycle, that means the RNA is evolvable in the cycle. We are now repeating the cycle to evolve the RNA spontaneously.

1P287	生命の始原系における原始タンパク質のアミノ酸配列についての仮説
	Investigation on the repetitive amino-acid sequences of native proteins and a hypothesis of the earliest proteins

Masahito Oka, (*Osaka prefecture university*)

Periodic proteins, which have the region composed of repetitive amino-acid sequences, are very interesting proteins from the viewpoint of molecular evolution. All repetitive amino-acid sequences were extracted from currently available databases. It is shown that the repetitive sequences generally contain Pro or Gly residue, and it is well known that Pro and Gly residues are characteristic ones among 20 naturally occurring amino-acid residues from the viewpoint of the conformational role of each amino-acid residue stabilizing the three-dimensional structure of proteins.Taking into account these points and going back the process of molecular evolution of proteins in the history of life, a new hypothesis can be derived on the question which has not been solved yet, that is, the earliest proteins had started their long evolution process from what kind of amino-acid sequence. An outline of the proposed hypothesis is as follows. The earliest proteins would be constructed by the repetitive amino-acid sequences.

1P288	対掌性の異なるアミノ酸の双極子モーメントに関する理論化学的研究
	Theoretical study on dipole moments of amino acids with different chirality

Shunsuke Mieda^{1,2}, Misako Aida^{1,2}, (¹*Graduate School of Science, Hiroshima University*; ²*Center for Quantum Life Sciences, Hiroshima University*)

Each amino acid except glycine has an asymmetric center at the alpha carbon: as a result, an amino acid can have either L- or D-type chirality. However, forms of most naturally-occurring amino acids are only of the L-form. Though various hypotheses have been proposed for the reason why amino acids have homochirality, there is still no conclusive explanation.

We focus on dipole moments of amino acids. The dipole-dipole interaction plays a significant role in predicting the reactivity or the stability between biological macromolecules such as proteins and DNA. Although the theoretical values of the dipole moments of all standard amino acids are available, dipole moments of amino acid residues in peptides have not been reported to our knowledge. Here, we calculate the dipole moments of all standard L- and D-amino acids with taking into account of polarization by main-chain of peptide. The term "dipole moment of amino acid" here indicates the dipole moment of an amino acid residue in a protein.

We find that the dipole moments of L-type enantiomer is larger dipole than that of D-type for most of amino acids. Larger dipole moment of an amino acid induces the larger stabilization effect by dipole-dipole interaction with other biological macromolecules. Thus, L-type enantiomer can induce larger dipole-dipole interaction than D-type. Therefore, most naturally-occurring amino acids have L-type chirality; in other words, the difference in the dipole moments between L- and D-type enantiomers may cause the homochirality.

1P289	モデル代謝系から構成される"生態系"の変動する環境に対する応答
	Evolution of ecological network of ideal metabolic networks under fluctuating environment

Akinori Awazu, (*Dept. of Math. and Life Sciences, Hiroshima University*)

Dynamic and statistical aspects of the class of simple ideal metabolic networks and the ecological network consists of such networks under the fluctuating resource supply are investigated. The ultimate goal of this investigation is to uncover the relation between two variational principles, the second law of thermodynamics and Darwin's theory of evolution.

We systematically construct simple reaction systems that describe ideal metabolic processes by thermodynamically reversible catalytic reaction networks consists of a few chemical components (catalysts) with inflow of resource chemical and outflow of waste chemical. First, we evaluate the metabolic performance of each reaction network by measuring the steady reaction flux and the response under strong and weak inflow of resource and the relaxation to thermodynamic equilibrium state under no supply of resource. Here, we found that the reaction networks involving the negative correlation of catalytic relations tend to exhibit the robust and high reaction flux.

Second, we consider the ecological system consists of the above ideal metabolic networks where they mutually suppress their activities with each other through the competition of a resource. By the simulation of this system under the fluctuating condition where the amount of the resource supply varies periodically, we found that which network can sustain its activity depends strongly on not only the period of the resource supply but also the history of the variations of the period.

1P290 原始地球の熱水環境における修飾海水からのアミノ酸の重合 Prebiotic oligomerization of amino acids with modified seawater under the hydrothermal environments in primitive earth.

Satoshi Shida, Ayaka Sugai, Hideaki Takehana, Eiichi Imai, Hajime Honda, (Department of Bioengineering, Nagasaki University of Technology)

In primitive earth, organic compound with low molecular weight must be oligomerized by the energy from natural environment to form some kinds of functional high molecular weight molecules. One of the plausible candidate should be the hydrothermal vent under deep sea where seawater containing a tough amount of metal ions in addition with amino acids, nucleic acids or other biologically-relevant molecules erupted from volcano at high temperature into cold seawater. We have been focusing on the process of quenching when the unstable large molecule with some biological function might have survived by soaking into cold aqueous milieu.

Modified seawater containing five types of amino acids with Fe, Cu, Mn, Mo, Ca, Na, K and Cl was reacted with a flow reactor simulating a hydrothermal vent. By using the reactor, we have succeeded to form glycine oligomers even in aqueous solution. The five amino acids were found to make several mixed oligomers detected by HPLC. Some of the produced oligomers have strong fluorescent emission which was found to increase as the development of a reaction within the reactor. Among the product molecule, some molecules which have the capacity to inhibit the degradation of glycine tetramers was found. The results should indicate that the environmental conditions of not only hydrothermal vent but also component of modified seawater would have a contribution for making a functional oligomers relevant for the emergence of life.

1P291 遺伝子の自己複製反応に与える反応場サイズ依存性 1H1325 Effects of cell size on internal self-replication of genetic information

Yohsuke Bansho¹, Norikazu Ichihashi², Tomoaki Matsuura², Tetsuya Yomo^{1,2,3}, (¹Frontier Biosciences, Osaka Univ.; ²Information Science and Technology, Osaka Univ.; ³ERATO, JST)

Living systems consists of many biochemical reactions performed in micro-scale compartments, cells. What effect does the size have on the internal reactions? We focused on the relationship between the size of cell-like compartment and the internal self-replication of genetic information.

We used w/o emulsions as cell-like compartments, which are small droplets of water dispersed in oil. First, we constructed a size-separating device using μ TAS technique that allowed us to separate the emulsions depending on the diameter into five fractions: 2, 3, 6, 12, and 16 μ m on average.

Second, we constructed a self-replication system of genetic information composed of a template RNA encoding RNA replicase and a cell-free translation system. RNA replicase was translated from the template RNA, and then replicated the template RNA. The self-replication, however, stopped after few rounds of replication because of the appearance of a parasitic RNA, a small RNA lacking the RNA replicase coding region and amplified enormously resulting in competitive inhibition of template RNA replication.

We found that the amplification of parasitic RNA was repressed and that of template RNA was slightly increased in smaller emulsions. These results were explained as follows. The parasitic RNA was confined in minor fractions of the droplets so that the other major fractions were free from the parasite, and

thus amplification of template RNA enhanced in the smaller emulsions. This result suggests that cell size can be a critical parameter of an internal reaction.

1P292 ドメインリンカーの構造と配列が構造ドメイン形成に及ぼす影響

Influence of Domain Linker Sequence and Structure on the Definition of Structural Domains.

Tepei Ebina, Shun Iwasaki, Yutaka Kuroda, (Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology, 12-24-16 Naka-machi, Koganei-shi, Tokyo 184-8588, Japan)

We report the influence of domain linker's structures and sequences on our definition of stable structural domains. In our definition, structural domains are domains that have no inter-domain contacts as evaluated using the atomic coordinates of the whole protein. The dataset used in the present study comprised 27 protein sequences that contain one domain linker separating two adjacent structural domains and that have at least one protein sequence homolog in the PDB with a fully conserved domain linker sequence.

The analysis demonstrated that the dataset can be divided into two parts: (1) 13 proteins in which the structural domain definition was conserved throughout the homolog PDB structures/sequences; and (2) 14 proteins in which the definition of structural domains depended on which PDB structure was used. We found that the amino acid composition of domain linkers in the first groups was different from that in the second groups. In particular we observed that prolines were over-represented in the second group. In addition, the RMSD of most domain linker regions in both groups were less than 0.5Å (average of ~0.2Å). Furthermore, we observed, for many proteins, that the RMSD among homologous domains in the second group were larger than in the first group. In the poster we will discuss possible implication of these observations on the inter- and intra-domain motions.

1P293 Modeling of 3R complexes from structure database

Kouta Mayanagi¹, Mihoko Saito², Takuji Oyama³, Yoshizumi Ishino¹, Tsuyoshi Shirai², (¹Kyusyu University; ²Nagahama Institute of Bioscience and Technology; ³Osaka University)

The computer system SIRD (Structure-Interaction Relational Database), which processes 2ry database of PDB by automatically classifying structures and interactions, has been developed. SIRD database can be used to infer protein complex structures by compiling the known structures. This system was applied to construct several 3R (Replication, Repair, and Recombination) complexes, for which no experimental structures were known, and the models from SIRD system were examined by solving the complex structures with X-ray crystallography and single-particle electron microscopy. The models revealed good agreement with the experimental structures. Especially, the models of ternary complex of DNA polymerase B-PCNA(clamp)-DNA predicted the presence of two different (polymerizing and editing) modes of this complex. A close inspection of the model suggested a conserved Glu residue of PCNA was important for the functional mode switch during DNA replication.

1P294 タンパク質-リガンド相互作用の共通性と多様性：混合正規分布推定による PDB からのデータマイニング Conservation of protein-ligand interactions: a data-mining of the PDB by using Gaussian mixture model

Kota Kasahara¹, Kengo Kinoshita^{2,3}, Toshihisa Takagi^{1,4,5}, (¹Grad. Sch. of Frontier Sci., Univ. of Tokyo; ²Grad. Sch. of Info. Sci., Tohoku Univ.; ³JST-BIRD; ⁴DBCLS; ⁵National Inst. of Genetics)

Proteins conduct versatile molecular functions through recognition of their specific ligands. Understanding of the specific ligand recognition of proteins is one of main subjects in the biophysics. Since the specificity is arisen from chemical and geometrical complementarities that stabilize a complex by inter-molecular interactions, analysis of the inter-molecular interactions based on 3D structure data of protein-ligand complexes is quite important.

In this field, structure comparison methods for binding sites of proteins have been studied. They have elucidated several structural patterns in the binding sites called "binding motifs" that play important roles on the ligand recognition. However, these methods cannot directly treat inter-molecular interactions since they have focused only on the protein structures. In order to investigate the patterns of interactions, both of protein and ligand structures should be considered.

We have carried out a data-mining for the inter-molecular interactions in protein-ligand complexes. Spatial distributions of interactions between protein and ligand fragments were collected from a database. And then, Gaussian mixture models were constructed from the spatial distributions in order to obtain the patterns of interactions as a Gaussian component. As a result, the interaction patterns that were conserved among several proteins have been obtained. Moreover, some of them were conserved among proteins with distinct